Huntingtin function during zebrafish

(Danio rerio) development

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**Statement of Originality**

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Tanya Lynn Henshall
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Tanya xox
**Abbreviations**

aa  amino acid
acridine orange  acridine orange hemi (zinc chloride) salt
amp  ampicillin
BCIP  5-bromo-4-chloro-3-indolyl phosphate
bh  basihyal (cartilage)
bp  base pairs
BDNF  brain derived neurotrophic factor
cDNA  complementary DNA
ch  ceratohyal (cartilage)
cMO  standard control morpholino
Ct  cycle threshold
DASPEI  2-(4-(dimethylamino)styryl)-N-ethylpyridinium iodide
DEPC  diethylpyrocarbonate
DF  degrees of freedom
DiI  1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiIC18(3))
DMF  dimethylformamide
DMSO  dimethylsulphoxide
DNA  deoxyribonucleic acid
dNTPs  deoxynucleotide triphosphates
dpf  days post fertilization
EDTA  ethylenediamine tetra-acetic acid
ef1a  elongation factor 1a
EGFP  enhanced green fluorescent protein
emx3  empty spiracles homeobox 3
ES  embryonic stem (as in ES cells)
EtBr  ethidium bromide
fgf8  fibroblast growth factor 8
GABA  γ-aminobutyric acid
HAP  huntingtin associated protein
HD  Huntington’s disease
GABA  γ-aminobutyric acid
hdMO morpholino antisense to zebrafish htt mRNA (as in hdMO1 and hdMO2)

HIP huntingtin interacting protein

htt huntingtin

hpf hours post fertilization

hs hyosymplectic cartilage

Kb kilobase pairs

kDa kilodalton

m Meckel’s cartilage

mcMO1 5 base mismatch of the hdMO1 antisense sequence

μM micromolar

ml millilitre

MLK2 mixed lineage kinase 2

mM millimolar

morpholino/MO morpholino oligonucleotide

MQ milli-Q

mRNA messenger RNA

NBT nitro blue tetrazolium chloride

ng nanogram

nl nanolitre

NMDA N-methyl-D-aspartic acid

nM nanomolar

ntl no tail

oligo oligonucleotide primer

omp olfactory marker protein

ORF open reading frame

OSN olfactory sensory neuron

otx2 orthodenticle homolog 2

p(3-7) pharyngeal arch (3-7)

PBS phosphate buffered saline

PBS-T PBS with 0.1% tween-20

pbx2 pre-B-cell leukemia transcription factor 2

PCR polymerase chain reaction

pmol picomoles

polyQ htt huntingtin with a pathogenic number of glutamine repeats
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>pq</td>
<td>palatoquadrate (cartilage)</td>
</tr>
<tr>
<td>PTU</td>
<td>1-phenyl-2-thiourea</td>
</tr>
<tr>
<td>qPCR</td>
<td>quantitative real-time PCR</td>
</tr>
<tr>
<td>RA</td>
<td>retinoic acid</td>
</tr>
<tr>
<td>REST/NRSF</td>
<td>RE-1 silencing transcription factor/neuron-restrictive silencer factor</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>rpm</td>
<td>revolutions per minute</td>
</tr>
<tr>
<td>SDS</td>
<td>sodium dodecyl sulphate</td>
</tr>
<tr>
<td>six1</td>
<td>sine oculis homeobox homologue</td>
</tr>
<tr>
<td>SSC</td>
<td>sodium chloride/sodium citrate buffer</td>
</tr>
<tr>
<td>TBS-T</td>
<td>tris-buffered saline with 0.1% Tween-20</td>
</tr>
<tr>
<td>TUNEL</td>
<td>terminal deoxynucleotide transferase (TdT)-mediated dUTP nick-end labeling</td>
</tr>
<tr>
<td>UTR</td>
<td>untranslated region</td>
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**Abstract**

Huntington’s disease shares a common molecular basis with eight other neurodegenerative diseases: expansion of an existing polyglutamine tract. In each case, this repeat tract occurs within otherwise unrelated proteins. These proteins show widespread and overlapping patterns of expression in the brain and yet the diseases are distinguished by neurodegeneration in a specific subset of neurons that are most sensitive to the mutation. It has therefore been proposed that expansion of the polyglutamine region in these genes may result in perturbation of the normal function of the respective proteins, and that this perturbation in some way contributes to the neuronal specificity of these diseases. The normal functions of these proteins have therefore become a focus of investigation as potential pathogenic pathways. Here, synthetic antisense morpholinos have been used to inhibit the translation of huntingtin protein during early zebrafish development. The results obtained show the effects of huntingtin loss-of-function on the developing nervous system, including distinct defects in morphology of the lateral line neuromasts, olfactory placode and branchial arches. The potential common origins of these defects were explored, revealing impaired formation of the anterior-most region of the neural plate as indicated by reduced pre-placodal and telencephalic gene expression with no effect on mid- or hindbrain formation. These investigations demonstrate a specific ‘rate-limiting’ role for huntingtin in formation of the telencephalon and the pre-placodal region, and differing levels of requirement for huntingtin function in specific nerve cell types.